REMARKS

Reconsideration of the present application is respectfully requested in view of the present amendment and the following remarks. Claims 16 and 17 are currently pending and under examination in the application. Entry of the present amendment is requested, by which claim 17 is canceled and claim 16 is amended to more particularly point out and distinctly claim the subject matter encompassed within the present embodiment. The present amendment is made solely to advance the prosecution of the application by placing it in condition for allowance, without acquiescence in any rejection and without prejudice to the prosecution of any surrendered subject matter in a related continuation, continuation-in-part or divisional application. Support for the present amendment can be found in the application as originally filed, for example, in the specification at page 7, lines 2-4; at page 5, lines 7-10; at page 2, lines 1-18; at page 5, line 25 through page 6, line 6; and elsewhere. No new matter is added by entry of the present amendment.

As explained in greater detail below, the applicant provides herewith additional evidence from the prior art in support of applicant's previous arguments of record for this application, to which entire record the PTO is also referred, and in view of which applicant requests reconsideration in the form of a "full and fair hearing" as called for, *inter alia*, by M.P.E.P. §706.07.

Briefly, it is submitted that:

- Contrary to the Examiner's assertion, evidence of record and knowledge in the prior art indicate that a formed gellan polymer sequesters Mg^{2+} cations at least as strongly as an EDTA concentration that is known to inhibit PCR reactions, such that the prior art recognized that even an *already-formed* gellan gel can *further* sequester additional Mg^{2+} cations. The prior art therefore teaches away from the instant embodiments, and the PTO errs by alleging otherwise.

- Gellan-enhanced PCR sensitivity could <u>not</u> have been predicted from the prior art. The PTO fails to provide even a scintilla of evidence that the prior art suggests a nucleic acid amplification reaction is capable of amplifying a lower level of a target nucleic acid

when gellan is present than can be amplified when gellan is absent. By alleging otherwise the PTO clearly employs impermissible hindsight in view of the instant application.

- Mere use of gellan as an electrophoresis medium in the prior art does not establish the obviousness of its ability to enhance PCR sensitivity. The PTO fails to satisfy the burden that it is *required* to meet, of showing why an allegedly inherent characteristic of the claimed subject matter *necessarily* flows from the prior art *and would have been recognized* by persons having ordinary skill in the art. On this point it is noted that the outstanding rejection is for obviousness, not novelty, such that when the subject matter of the claim is considered as a whole, as is proper, the presently claimed composition did not exist in the prior art and thus the allegedly inherent property cannot have been recognized in a prior art composition.

REJECTION UNDER 35 U.S.C. § 103

Claims 16 and 17 stand finally rejected under 35 U.S.C. § 103(a) for alleged obviousness over Mitra et al. (*Nucleic Acids Research* 27(24)e34: i-iv, 1999) in view of Cole et al. (*BioTechniques* 26:748-756, 1999). The PTO asserts that Mitra et al. teach an amplification reaction in polymerized acrylamide, and that Cole et al. teach the use of gellan as a separation medium for electrophoresis. The PTO then asserts that it would have been obvious to substitute the acrylamide of Mitra et al. for the gellan of Cole et al. to arrive at the presently claimed subject matter, and alleges that a person skilled in the art at the time of invention would have been motivated to make such a substitution since gellan (i) is an alternative gel material that allows easy DNA recovery, (ii) requires low concentrations for gel formation, and (iii) reversibly forms gels.

Applicant traverses these grounds for rejection and submits that the instant claims satisfy the requirements of 35 U.S.C. §103. The presently claimed subject matter is directed to a nucleic acid amplification reaction mixture for use in nucleic acid amplification of enhanced sensitivity, comprising water, gellan at a concentration above 0.005 wt% based on the weight of water, a DNA polymerase, dNTPs, and a target nucleic acid, wherein the nucleic acid amplification reaction mixture is capable of amplifying a lower level of the target nucleic acid when gellan is present than can be amplified when gellan is absent. As a first matter, the

rejection of claim 17 will be rendered moot upon entry of the amendment submitted herewith, in which claim 17 is canceled.

Clear teachings of the unexpected ability of gellan to enhance the sensitivity of a nucleic acid amplification reaction appear for the first time in the present application as originally filed, for example, in the specification at page 7, lines 2-4; at page 5, lines 7-10; at page 2, lines 1-18; at page 5, line 25 through page 6, line 6; and elsewhere.

For reasons given herein, and also for reasons previously made of record, the prior art fails in any way to teach or suggest the use of gellan to enhance the sensitivity of a nucleic acid amplification reaction, and if anything the prior art teaches away from inclusion of gellan in a nucleic acid amplification reaction mixture. The Examiner's allegations that gellan (i) is an alternative gel material that allows easy DNA recovery, (ii) requires low concentrations for gel formation, and (iii) reversibly forms gels, are therefore beside the point, because none of these properties would have provided the ordinarily skilled person with the reasonable expectation of successfully predicting that inclusion of gellan in a nucleic acid amplification reaction would result in a reaction mixture that is capable of amplifying a lower level of target nucleic acid than would be the case if gellan were not present.

The PTO therefore impermissibly employs hindsight in its assertion of the obviousness rejection. In this regard, based on the prior art teachings, which are limited to descriptions of gellan as an electrophoresis medium suitable for nucleic acids, gellan would be no more expected to substitute for acrylamide in the reaction of Mitra et al. than would any other known media for nucleic acid electrophoresis, such as agar, agarose, starch, polydextran, or other media. The PTO fails to provide evidence or reasoning as to why the skilled person would have expected predictably and successfully to arrive at a nucleic acid amplification of enhanced sensitivity by including gellan, absent the disclosure of the present application.

Additionally, the method of Mitra et al. depends on an immobilized primer that is covalently and irreversibly incorporated into the acrylamide gel by free radical-mediated chemical crosslinking (page ii), where such incorporation of the primer is "crucial" (page v, first full paragraph) to obtain the reduced size PCR colony ("polony") on which the method of Mitra et al. relies. These essential features of Mitra et al. teach away from the use of reversibly

assembled gellan, where the skilled person would recognize that the permanently assembled, covalent copolymerization of primers in Mitra et al. would not be compatible with the ionic, non-covalently assembled gellan of Cole et al. Moreover the PTO errs in alleging that gellan of Cole and acrylamide of Mitra are interchangeable equivalents, because it has been settled that such an assertion of equivalency must be a functional or mechanical equivalency that is recognized by the prior art for the same purpose. M.P.E.P. §2144.06, citing *In re Ruff*, 256 F.2d 590 (CCPA 1958). On this point, and for reasons given herein, the prior art fails to recognize that a nucleic acid amplification reaction mixture is capable of amplifying a lower level of target nucleic acid when gellan is present than can be amplified when gellan is absent, and the PTO fails to present evidence or reasoning that any motivation can be found in the prior art to use gellan for the same purpose, *i.e.*, to obtain an amplification of enhanced sensitivity according to the presently claimed subject matter. The PTO therefore errs by alleging that it would have been obvious merely to substitute the gellan of Cole et al. for the acrylamide of Mitra et al., because the PTO fails to establish that such a substitution would be for the same purpose.

Contrary to the Examiner's allegation that an already-formed gellan gel is not capable of further Mg^{2+} sequestration (Action, at page 4), submitted herewith is evidence from the prior art showing that an already-formed gellan gel is capable of further chelating Mg^{2+} ions, which would teach away from the presently claimed subject matter by suggesting that gellan would inhibit a nucleic acid amplification reaction as efficiently as does EDTA. The person having ordinary skill in the art therefore could not reasonably have expected *successfully* to amplify a nucleic acid in a gellan gel merely by substituting gellan for the acrylamide of Mitra et al.

Enclosed for the Examiner's convenience is a copy of Chandrasekaran (*Adv Exp Med Biol*. 302:773-84, 1991). Chandrasekaran used x-ray fiber diffraction to characterize the structure of polycrystalline gellan and further carried out computer modeling of the influence of calcium ions –which, like Mg^{2+}, are divalent cations-- on gellan aggregation and native gellan morphology. Chandrasekaran describes the contribution to gellan structure of water molecules and of potassium (monovalent) cations to form gellan gels.

Significantly, Chandrasekar further describes (Figure 4 and pages 779-781) how in a formed gellan gel a single calcium (divalent) cation can replace the monovalent potassium cations to crosslink gellan in the gel *more strongly*, noting that "gelation could take place at a relatively low concentration of calcium, *compared to that in the presence of potassium.* This is completely consistent with reports from solution studies. (citations omitted)" (Chandrasekar, page 781, emphasis added). It will be noted that Doner et al. (1991 *Biotechnol. Techniques* 5:25, of record) disclose sequestration by gellan of divalent magnesium cations and of divalent calcium cations.

It is therefore clear from Chandrasekar and Doner et al. that at the time of filing the present application, the person having ordinary skill in the art would have expected even an already-formed gellan gel to be capable of incorporating divalent cations, and would bind divalent cations more strongly than it does monovalent potassium cations, as demonstrated by Chardrasekar. Hence, the PTO errs in asserting that "[w]hile chelating agents can solubilize a formed gellan gel, this does not demonstrate that addition of further divalent cations are taken up by the gellan gel." (Action, page 4) As discussed above, the prior art contains clear evidence to the contrary.

The prior art also teaches away from the present subject matter because in the prior art can be found evidence that the skilled person, at the time of filing the present application, would reasonably have believed that gellan would inhibit a nucleic acid amplification reaction as efficiently as EDTA.

In this regard, for example, Cole et al. (of record) teach that a concentration of 2 mM EDTA was required to fully chelate calcium to free DNA from a formed gellan gel (*see* page 756, column 1, second full paragraph). From this teaching, it is submitted the skilled person would reasonably have concluded that formed gellan gels sequester divalent cations almost as strongly as 2 mM EDTA, since that concentration was needed to fully chelate the divalent cations. It is submitted further that the skilled person would certainly have reasonably believed that where 2 mM EDTA was required to fully chelate calcium, then lowering the amount of this agent to only one-half the required concentration, *i.e.,* to 1 mM EDTA, would have been considered insufficient to chelate calcium from a formed gellan polymer.

The skilled person would further have reasonably appreciated that even 1 mM EDTA, *i.e.*, roughly one-half the concentration needed to fully chelate calcium and free DNA from a formed gellan gel according to Cole et al., was still inhibitory for nucleic acid amplification, as taught, *e.g.,* by Kreader (*Applied and Env Microbiology* 62:1102-1106, 1996; *see* page 1103, column 2, first full paragraph; and Figure 1). Hence, given the teachings (i) of Cole et al. that gellan polymers sequester divalent cations more strongly than 1mM EDTA (*i.e.*, where double that EDTA concentration is required to chelate the divalent cations in a gellan gel according to Cole et al.), and (ii) that the chelating agent EDTA is certainly capable of inhibiting nucleic acid amplification reactions when present at a concentration of only 1 mM according to Kreader , it is submitted that given the prior art at the time of filing the instant application, the person having ordinary skill in the art would reasonably have believed that gellan polymers would inhibit nucleic acid amplification reactions and could do so because of their affinity for divalent cations (*e.g.*, Mg^{2+}), particularly where the gellan would be expected to be present in excess relative to other reaction components. On this point see also, *e.g.*, specification at page 5, line 25 through page 6, line 6.

It is therefore respectfully submitted that based on the state of the art at the time of filing the present application, a person having ordinary skill would not reasonably have expected successfully to prepare a nucleic acid amplification mixture comprising gellan at a concentration above 0.005 wt% based on the weight of water. In this regard, and for reasons given herein, the prior art clearly teaches away from the subject matter of the instant claim.

Furthermore, and contrary to the Examiner's allegation, the advantages that are obtained by making the presently claimed combination cannot be "inherently present" in the prior art where the presently recited nucleic acid amplification reaction mixture comprising gellan did not exist in the prior art. Enhanced sensitivity of nucleic acid amplification reactions performed in the presence of gellan is disclosed for the first time in the present application, such that the PTO employs impermissible hindsight in its allegation that the skilled person would have been motivated to substitute gellan for the acrylamide of Mitra et al. Applicant has previously noted that under *KSR v. Teleflex*, the PTO must show that the claimed combination would have yielded nothing more than results that the skilled person would have found predictable. Here,

the PTO fails to provide any evidence that from the prior art, and without impermissibly applying the teachings of the present application, successful nucleic acid amplification at all, and much less amplification with enhanced sensitivity, could have been predicted to result from the inclusion of gellan in a nucleic acid amplification reaction mixture.

In this regard, it is well settled that the claimed invention must be considered as a whole (*KSR International v. Teleflex Inc.*, 127 S. Ct. 1727, 1734 (2007); *Kimberly-Clark Corp. v. Johnson & Johnson*, 745 F.2d 1437, 1448 (Fed. Cir. 1984); M.P.E.P §2141.02) such that, as discussed above, the presently claimed subject matter relates to enhanced sensitivity conferred upon a nucleic acid amplification reaction by the presence of gellan as recited, in a manner that is entirely unforeseen by the prior art. M.P.E.P. 2141.02 provides that:

> Obviousness cannot be predicated on what is not known at the time an invention is made, even if the inherency of a certain feature is later established. *In re Rijckaert*, 9 F.2d 1531, 28 USPQ2d 1955 (Fed. Cir. 1993).

Accordingly, the PTO has failed to meet its burden of establishing that the subject matter of the presently claimed invention is inherently present in the prior art, particularly where, as noted above, there is absolutely no adumbration of the sensitivity-enhancing properties of gellan anywhere in the prior art and if anything, the prior art teaches away from the use of gellan in nucleic acid amplification.

M.P.E.P §2112 provides that:

> In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. *Ex parte Levy*, 17 USPQ2d 1461, 1464 (BPAI 1990) (emphasis in original).

Accordingly, the applicant submits that the burden remains with the PTO to supply the requisite basis in fact and/or technical reasoning, where it is submitted that mere conjecture on the part of the PTO (that the skilled person would have been motivated to substitute gellan of Cole et al. for acrylamide of Mitra et al.) does not suffice as a finding that the prior art references, whether each taken alone or in combination, contain a disclosure that renders obvious the presently claimed invention. Furthermore, the PTO has offered no evidence making

clear that "the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill." (*Continental Can Co. USA v. Monsanto Co.,*948 F.2d 1264, 1268, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991) (M.P.E.P. § 2131.01 (III), emphasis added).

In this regard, and for reasons also discussed elsewhere herein, selection for use in nucleic acid amplification of gellan over any other electrophoretic media has absolutely no basis anywhere in the prior art, absent impermissible hindsight reconstruction in view of the instant application. The United States Supreme court recognized that "hindsight bias" and "ex post reasoning" are inappropriate in a determination of obviousness (*KSR v. Telflex*, 127 S. Ct. at 1742), and that a "combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results" (127 S. Ct. at 1739). For reasons given herein and previously made of record in the present application, the result of enhanced sensitivity in a nucleic acid amplification reaction mixture that comprises above 0.005 wt% gellan was unpredictable where, in view of the state of the art, a person having ordinary skill would not have had the reasonable expectation of successfully practicing nucleic acid amplification in the presence of gellan. The Federal Circuit has recently reiterated a similar analysis concerning the nonobviousness of a claimed combination in view of its unpredictability. See *Sanofi-Synthoelabo, Inc. v. Apotex, Inc.*, 89 USPQ2d 1370 (2009, decided December 2008).

In view of the foregoing it is submitted that the PTO fails to meet its burden of establishing a *prima facie* case of obviousness with respect to the presently claimed subject matter. *See In re Mayne*, 104 F.3d 1339 (Fed. Cir. 1997).

Accordingly, the applicant submits that all of the claims in the application are allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC

/Stephen J. Rosenman/

Stephen J. Rosenman, Ph.D.
Registration No. 43,058

SJR:rp

Enclosures:

Chandrasekaran (*Adv Exp Med Biol*. 302:773-84, 1991).
Kreader (*Applied and Env Microbiology* 62:1102-1106, 1996)

701 Fifth Avenue, Suite 5400
Seattle, Washington 98104
Phone: (206) 622-4900
Fax: (206) 682-6031

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